PAN-PACIFIC ENTOMOLOGIST
64(1), 1988, pp. 17–22

Larval Development and Leafmining Activity of *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae)

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Abstract.—The larval development of *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) was investigated using chrysanthemum (*Dendranthema grandiflora* Tzvelev, cultivar 'Hurricane') as the host. With a mean temperature of 28.4°C, median duration was 0.85, 1.23, and 1.42 days, respectively, for first, second, and third instars. Third instars mined the greatest area, ca. 4- and 30-fold that of second and first instars, respectively. Measurements (length) of the cephalopharyngeal skeleton followed the Brooks-Dyar rule of geometric growth, with a growth ratio of 2.03, and mean skeletal lengths (mm) were 0.10 (firsts), 0.17 (seconds), and 0.27 (thirds). Both serpentine and blotchlike mines were observed. Mean mine lengths of serpentine mines (mm) from the point of egg hatch to larval location were 5.28, 9.85, and 21.57 for first, second, and third instars, respectively. Third instars mined at a rate that was ca. 9.4-fold that of first instars and 4.5-fold that of seconds; the mining rate of second instars more than doubled that of firsts.

A serpentine leafminer, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) has been the focus of considerable research during the past 5 years (Parrella and Robb, 1985) as a consequence of its sudden rise to major world-wide pest status on numerous ornamental and vegetable crops (Lindquist, 1983). Several recent studies have reported selected aspects of the biology of *L. trifolii* (Charlton and Allen, 1981; Parrella et al., 1983; Mora and Mosquera, 1984; Bodri and Oetting, 1985; see Parrella [1987] for a thorough review). However, few of these studies examined the larval development of this leafminer in detail. Often no discrimination of larval instars has been made; many studies report larval development as if there were only one instar, and sometimes even combine egg and larval development.

Accurate larval development data are necessary for a more thorough understanding of the biology of *L. trifolii* on chrysanthemum and other hosts. Furthermore, our studies and others have emphasized the use of parasites for control of this leafminer on ornamentals (Jones et al., 1986; Woets and van der Linden, 1985; see Minkenberg and van Lenteren [1986] for a thorough review). Corollary studies with these parasites (i.e., searching behavior, instar preference with respect to oviposition and host feeding, factors influencing sex ratio, etc.) necessitate detailed data on larval development of *L. trifolii.*

This study was undertaken to evaluate the larval development and leafmining activity of these leafminers in chrysanthemum. In particular, we were interested in documenting instar size, stadial duration (Jones, 1978), and the amount of leaf area mined by each instar.

Materials and Methods

Eighty rooted chrysanthemum plants (cultivar 'Hurricane') were grown in 9.67 cm^2 pots on raised benches in the greenhouses at the University of California, Riverside. Standardized chrysanthemum plants (same age and size) were used to avoid the bias that may be associated with larval development on leaves of different ages on the same plant. The soil mix used consisted of 1 part vermiculite, 1 part peat, and 2 parts soil and the plants were given Osmocote® (14-14-14) (2.5 g/pot) ca. 4 days after planting. Supplemental lighting maintained a 13:11 photoperiod. Plants were grown for 5 weeks, then pinched to 10 leaves before exposure to colonies of *L. trifolii.* This colony is maintained on chrysanthemum (cultivar 'Hurricane,' 'Florida Marble,' 'Blue Chip') and wild flies from commercial chrysanthemum greenhouses in California were added at approximately monthly intervals. Details of colony maintenance are provided elsewhere (Parrella, 1983; Parrella et al., 1983).

Plants were exposed to a leafminer colony (ca. 2,000 flies) for 1/2 hour at 12 Noon on 18 November. All subsequent larvae hatched from eggs laid during this period. After exposure plants were placed in an environmental chamber of 28.4 ± 0.06°C ($\bar{x}$ ± SE), 13:11 L:D photoperiod and 60% RH. Plants were observed for egg hatching and larval development at 6-h intervals (1/4 days) beginning on day 2.5. At each 6-h interval, through 12 Noon, 24 November (6 days), 10 leaves with nonintersecting mines were removed from plants and one mine per leaf was selected and photographed, using a Wild® photomacroscope at 8× or 16×. Although larvae of *L. trifolii* prefer to mine the upper palisade misophyll of a chrysanthemum leaf (Parrella et al. 1985), they occasionally mine the spongy mesophyll. Only larvae mining the upper palisade meosphyll were used. Large third-instar mines sometimes required several photographs to encompass the entire mine. These photographs were joined together for analysis.

After photographing, larvae were removed from the leaves and placed in dilute alcohol for later mounting on microscopic slides in Hoyer's mounting media. The cephalopharyngeal skeleton of each larva was then measured using a microscope fitted with an ocular micrometer. From the photographs, serpentine mine length was calculated as was the width of each mine at the location of the larva. Mine area was determined using a TRS® 80 computer with High Pad® digitizing software; as the area encompassing a mine is outlined, the area is calculated.

Statistical Analysis

The Brooks-Dyar rule of geometric growth (Hutchinson and Tongring, 1984; Daly, 1985) was used to determine the number of larval instars. The Ln (cephalopharyngeal skeletal length) [dependent variable] was regressed against the presumed instar number (independent variable) (Ray, 1982).

Because larvae were killed for instar determination at 6-h intervals, the total development time of and area mined by any one larva from egg hatch to pupation was not recorded. Duration of each larval stadia was determined by linear regression of the proportion of successive instars (dependent variable) against time (independent variable), thus providing the median (e.g. 50% firsts—50% seconds) time of transition between instars. At such time the preceding instar population will also have mined its maximum before transition. Therefore, by linear regression of log (area mined) against time and selecting the area mined at time of first instar

Table 1. Duration, area mined, and size of the cephalopharyngeal skeleton for the instars of *L. trifolii*.

Instar	*Stadial duration*[1] *(days)*	*Area mined*[2] *(cm^2)*	*$\bar{x}$ cephalopharyngeal size (mm) (± SE)*
First[1] N = 28	0.85	0.07	0.100 ± 0.0
Second N = 48	1.23	0.29	0.172 ± 0.0
Third N = 50	1.42	1.36	0.267 ± 0.0

[1]Calculations were initiated after an egg stage of 2.5 days.
[2]Method of calculation explained in text.

transition, the approximate area mined during the first larval stadium was obtained. A similar procedure was followed with each instar. At this point, subtracting the area mined by first instars from seconds and second instars from thirds, an approximate area mined per instar was calculated.

To determine rates of mining between instars, regression analysis was used where mean mine area of each instar (dependent variable) was regressed against time (dependent variable). The slope of the lines gives the rate at which instar mined a leaf.

Results and Discussion

The presence of 3 larval instars within the leaf was confirmed. Regression of the natural log of mean cephalopharyngeal skeletal lengths against presumed instar number was represented by $Y = 5.64 + 2.03\times$, $R^2 = 0.99$. Mean cephalopharyngeal skeletal lengths (mn) were 0.10, 0.17, and 0.26 for first, second, and third instars, respectively (Table 1). These are pictured in Mora and Mosquera (1984). Cephalopharyngeal skeletal size increased by a constant factor (slope = 2.03) which follows the Brooks-Dyar rule of geometric growth (Hutchinson and Tongring, 1984; Daly, 1985).

The duration of the first larval stadia was less than one day while second and third stadia required greater than one day (Table 1). The area mined increased with each instar, with the third instar mining ca. 4- and 20-fold the area mined by the second and first instars, respectively. This is considerably different from data reported by Fagoonee and Tory (1984). Differences in host plant as well as temperature could possibly explain this discrepancy. In addition, Fagoonee and Tory (1984) did not report how instar determination was made nor how often larval development was checked.

In the chrysanthemums and other hosts (Suss et al., 1984), *L. trifolii* does not always made serpentine mines; occasionally blotchlike mines are observed. The length of the mine and, in particular, mine width, are affected by the type of mine created. Blotch mines are generally shorter than serpentine mines (i.e., the larva moves a smaller distance in the leaf over the same time period); however, they are usually much wider (Table 2). The distance traveled by a larva approximately doubled with each successive instar. The amount of leaf area mined per day for each

Table 2. Selected measurements ($\bar{x} \pm$ SE mm) of the larval mining behavior of *L. trifolii*.

Instar	*Serpentine mine width*[1]	*Serpentine mine length*[1]	*Widest point of a blotch mine*	*Blotch mine length*
First	0.264 ± 0.012 (17)[b]	5.28 ± 0.36 (28)	1.017 ± 0.090 (14)	0.746 ± 0.097 (9)
Second	0.492 ± 0.030 (27)	9.85 ± 0.74 (46)	1.676 ± 0.144 (35)	0.852 ± 0.084 (15)
Third	1.126 ± 0.056 (37)	21.57 ± 1.28 (43)	3.78 ± 0.28 (28)	3.049 ± 0.244 (12)

[1]Measured at the location of the larva.
[2](N).

instar (Fig. 1) clearly shows that the third instar develops the most rapidly and consumes the greatest amount of leaf material compared to the other instars. Linear regression of mine area of each instar against time (Fig. 1) produced $Y = 0.0074 + 0.0385\times$, $R^2 = 0.96$ for first instars, $Y = -0.0033 + 0.08\times$ for second instars, $R^2 = 0.89$ and $Y = -0.0319 + 0.365\times$ for third instars, $R^2 = 0.64$. Based on the slopes of the regressions, the third instar creates a mine ca. 9.4-fold the rate of a first instar and ca. 4.5-fold that of a second instar. The second instar approximately doubled the mining rate of a first instar.

The ability of a parasite to find the larva of a leafminer may be related to the distance between its antennae among other factors (Sugimoto, 1977); adult females continually tap a leaf with their antennae in search of a mine. Parasites may be able to locate mines that have a diameter smaller than the width between their antennae by coming upon a mine perpendicularly. However, they may be unable to orient along this narrow mine when initiating a search for the leafmining larva. Thus, size of the mine created by leafmining larvae may influence the degree of susceptibility of each instar. Knowledge of the area mined by each instar may be a useful predictor (together with detailed studies on parasite searching, and other behaviors) of whether a particular instar will be attacked. Such information (together with intrinsic rate of increase, overall searching efficacy, ease of mass rearing, etc.) may be important in making decisions as to what parasite species may be the best to use for biological control of *L. trifolii.* Those parasites attacking younger instars would be preferred in both ornamental and vegetable crops. In ornamental crops they would stop mine development at an early stage thus reducing aesthetic injury and in vegetable crops small mines are likely to make less of an impact on plant yield compared to larger mines.

Acknowledgments

The critical review of John Sanderson (Dept. of Entomology, Cornell University) was greatly appreciated. This research was supported in part by the American Florists Endowment. The generous donation of chrysanthemums from Yoder Brothers of Florida, Inc. and the California Plant Corporation is gratefully acknowledged.

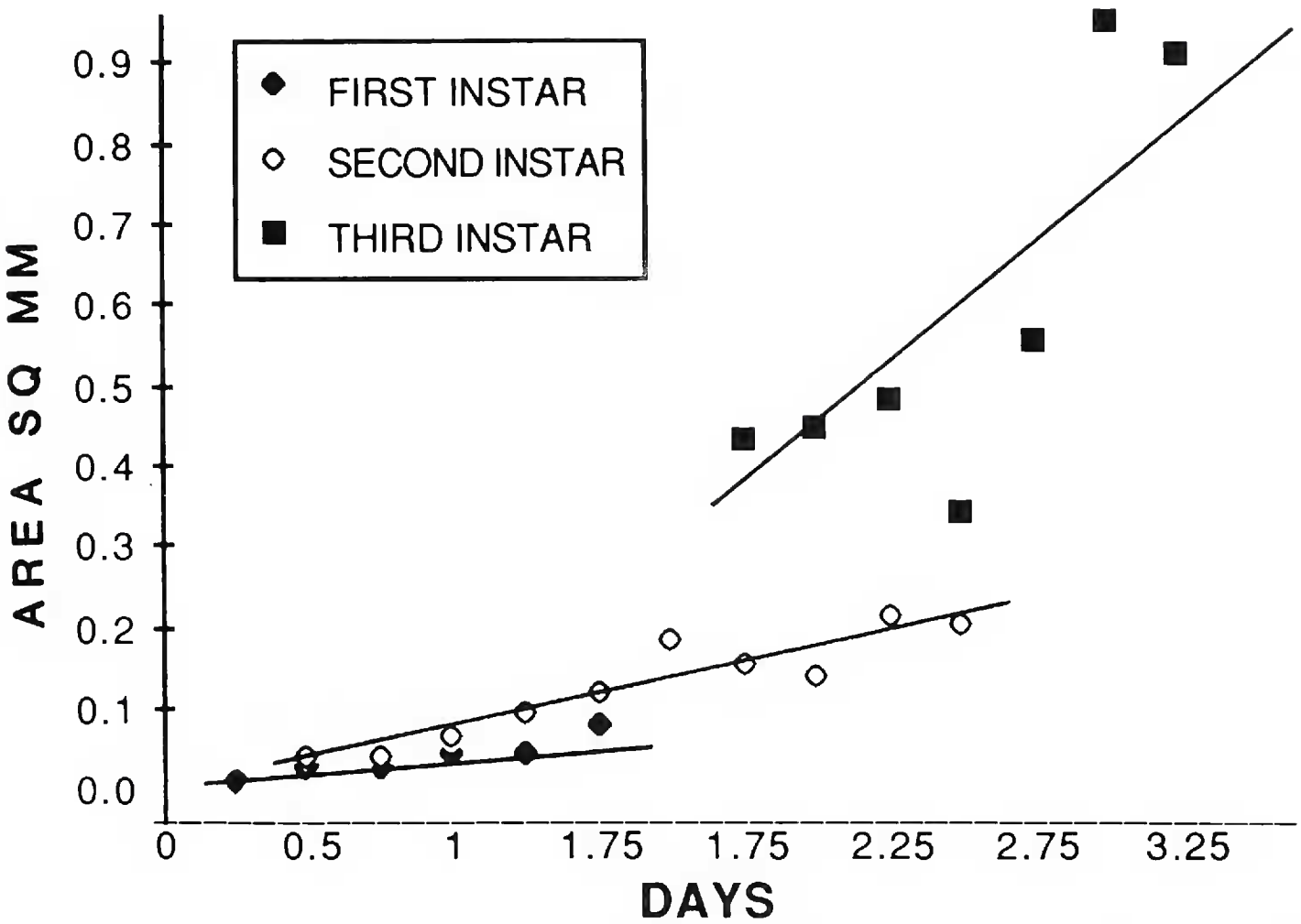

Figure 1. Amount of chrysanthemum leaf area mined and mining rate through time for each larval instar of *L. trifolii* at 28.4°C. Solid lines represent least squares linear regression between areas mined and time for each instar (see text).

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